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The importance of Val-157 hydrophobic interaction for papain inhibitory activity of an epoxysuccinyl amino acid derivative

A structure–activity relationship based on the crystal structure of the papain–E-64-c complex

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Based on the crystal structure of the papain–E-64-c complex, 3-dimensional binding modes of a series of epoxysuccinyl amino acid derivatives to the papain active site have been constructed and the structure–inhibitory activity relationship has been analyzed using the accessible surface area and nonbonded energy parameters. The result indicates the importance of the hydrophobic interaction between the amino acid side chain of the inhibitor and the papain Val-157 residue for revealing the potent inhibitory activity.

Papain; Epoxysuccinyl amino acid derivative; Inhibitory activity; Quantitative structure–activity relationship; Hydrophobic interaction

1. INTRODUCTION

The importance of the physiological roles of thiol proteases has achieved wide recognition [1–3]. Potent thiol protease inhibitors serve not only as a powerful tool in the clarification of the physiological roles of the proteases, but also as a therapeutic drug for diseases caused by abnormal elevation of proteolytic activity [4,5].

Since E-64 (**1**) (see Fig. 1) was isolated from a culture of *Aspergillus japonicus* as a potent irreversible inhibitor for thiol proteases such as papain, cathepsin and Ca^{2+}-activated neutral protease [6,7], various kinds of derivatives have been examined with the aim of developing a clinically usable drug. During these processes, a series of epoxysuccinyl amino acids (EAA, **3**) were synthesized and their inhibitory activities (ID_{50}, in nmol) against papain were examined (see Table I) [8]. We recently carried out an X-ray crystal structure analysis of the papain–E-64-c (**2**) complex: $R = 15.9\%$ using 10168 reflections within a resolution of 2.1 Å. Judging from the similarity between the chemical structures of EAA (**3**) and E-64-c (**2**) (see Fig. 1), the binding mode of the EAA main chain to papain could be the same as that of E-64-c. Thus, the differences in inhibitory activities measured for EAA derivatives may be due to the differences in their amino acid side chains.

This paper deals with the importance of hydrophobic interaction of the EAA side chain with the papain active site, especially with the Val-157 residue, elucidated by QSAR (quantitative structure–activity relationship) analyses using parameters of van der Waals nonbonded energy and accessible surface area (ASA) [9].

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2. EXPERIMENTAL

2.1. *Tertiary structure construction of the papain–EAA complexes*

The tertiary structures of the papain–EAA complexes were constructed based on the X-ray crystal structure analysis of the papain–E-64-c complex (D. Yamamoto et al., unpublished data). Construction of iterative visual models was carried out with IRIS3000 color graphics using the program MMS (Molecular Design, CA). The EAA main chains were fitted to the papain active site so as to assume the same interaction mode as the E-64-c. Each amino acid side chain of EAA was fitted to avoid any steric short contact with papain, adjusted to achieve the most suitable conformation [10], and then subjected to energy minimization using the program AMBER [11] until the energy gradient was smaller than 1.0 kcal/mol per Å; atoms moved in minimizations were restricted to the amino acid side chains of EAA derivatives.

2.2. *Calculations of accessible surface area and nonbonded energy*

ASA is defined as the area on the surface of a sphere radius R on each point of which the center of a solvent molecule can be placed in contact with the atom without penetrating any other atoms of the molecules [9] and has been used to estimate the matching degree between the enzyme and substrate or inhibitor. Difference accessible sur-

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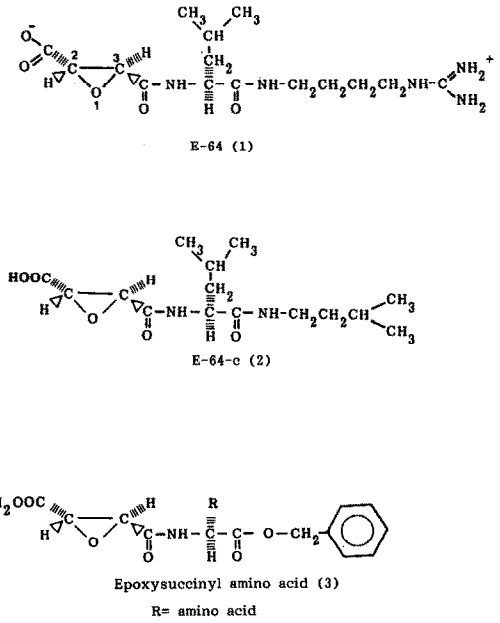

Fig. 1. Chemical structures of E-64 (1), E-64-c (2) and an epoxysuccinyl amino acid derivative (3).

face area (DASA), defined as the difference between the ASAs of papain and of papain+EAA derivative, was used to estimate the degree of the interaction of the EAA derivative with the papain.

However, since the ASA does not take the effect of atomic repulsion into consideration, the nonbonded atomic attractive and repulsive energies ($V_{nb} = V_{att} + V_{repul}$) between the papain and EAA derivative were calculated by the Lennard-Jones equation using the standard data set [12]. Amino acid residues of papain included in the calculations were within 10 Å from the EAA amino acid Cα atom.

Table I

Papain inhibitory activities (ID_{50}, in nmol) of EAA derivatives and their DASAs (Å^2) for total molecules and for Val-157 residues, and V_{nb} and V_{repul} energies (kcal/mol)

R	ID_{50}	DASA (total)	DASA (Val-157)	V_{nb}	V_{repul}
Gly	142.90	193.13	3.92	−20.20	9.78
Val	9.95	224.87	18.18	−22.50	10.95
Leu	9.93	230.78	19.82	−23.17	12.08
Ile	4.30	227.52	22.66	−23.33	11.41
Phe	24.66	233.86	30.60	34.20	80.72
Tyr	24.70	235.07	31.40	38.71	85.87
$-CH_2CH_3$	29.60	214.39	12.84	−22.07	11.34
$-(CH_2)_2CH_3$	17.09	224.39	16.17	−22.90	11.97
$-(CH_2)_3CH_3$	14.03	234.86	17.26	−23.52	12.53

2.3. *QSAR analyses*

In developing the correlation equations, the QSAR program [12] was used using the linear least-squares regression method.

3. RESULTS AND DISCUSSION

Papain inhibitory activities (ID_{50}) of EAA derivatives, the DASAs of each EAA molecule for the total papain molecule and for the Val-157 residue only, and V_{nb} and V_{repul} energies are summarized in Table I. A stereoscopic view of the papain–EAA(Ile) interaction mode is shown in Fig. 2.

The covalent bond formation of the epoxy ring C_2 atom to the papain Cys-25 S_γ atom with *R*-configuration was already established [13]. As a result

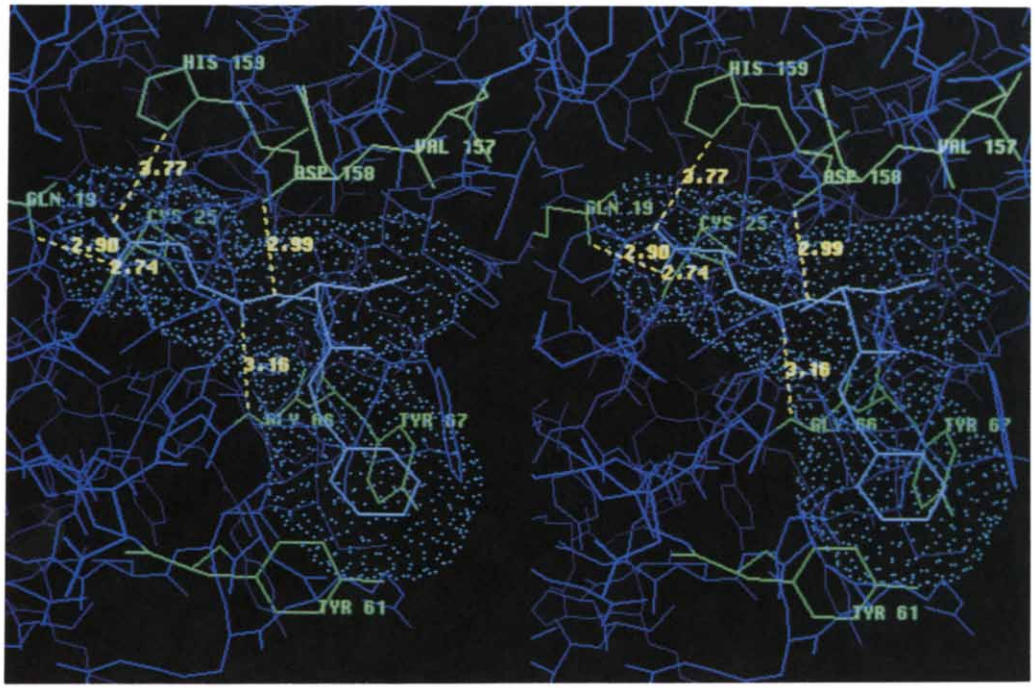

Fig. 2. Stereoscopic view of the binding mode of EAA(Ile) with the papain active site. Highlighted in light blue is the EAA(Ile) molecule. The dot circles show the van der Waals sphere of EAA(Ile). Possible hydrogen bonds and electrostatic interactions are shown by broken lines. Respective amino acid residues composing the papain active site are also labelled. The ethyl ester of EAA is proven to be replaced by the carboxylic acid form in the cell.

Table II

Correlation coefficients of QSAR parameters and correlation equations for inhibitory activities

(1) Linear correlation coefficient of each QSAR parameter with inhibitory activity

	DASA (total)	DASA (Val-157)	V_{nb}	V_{repul}
r	0.6899	0.4756	−0.1955	−0.1503

(2) Correlation equations for inhibitory activities

		r
Eqn 1: $\log(1/ID_{50})$ =	$-8.7916 + 0.0331$ DASA(total) $- 0.0098\ V_{nb}$	0.8758
	(1.4151)[a] (0.0062) (0.0029)	(0.1920)
Eqn 2: $\log(1/ID_{50})$ =	$-2.2930 + 0.0808$ DASA(Val-157) $- 0.0193\ V_{repul}$	0.9965
	(0.0341) (0.0023) (0.0006)	(0.0333)

[a] The values in parentheses represent the estimated standard deviations

of model construction, the main chain of the EAA could be stably fixed by virtue of: (i) the hydrogen bonds of O5...Gly-66 N, N6...Asp-158 O, O17...Gln-19 $N^{\epsilon 2}$ and O17...Cys-25 N; (ii) the electrostatic interaction of O18...His-159 $N^{\delta 1}$; and (iii) the hydrophobic interactions of benzyl ester...Tyr-61 and Tyr-67. Therefore it is obvious that the difference in the inhibitory activity observed in EAA derivatives is due to the interaction mode of the EAA side chain with the papain active site, especially with the Val-157 residue.

As indexes to represent the matching degree of the EAA derivative with papain, DASA, V_{nb} and V_{repul} values were considered. While DASA represents the contact area between the EAA and papain molecules, V_{nb} or V_{repul} reflects the energetic instabilities accompanying the short contacts of two interacting molecules. Thus these two parameters could act as functions complementary to each other.

The results of QSAR analyses are given in Table II. Although no significant correlation with the activity was observed for any of the used QSAR parameters, their linear combinations give good correlation equations. As might be expected from Eqn 1, respective minus and plus contributions of DASA and V_{nb} parameters to the inhibitory activity ($\log(1/ID_{50})$) imply that an amino acid side chain such as Ile, Leu or Val corresponds to the most suitable space for the tight binding of EAA with papain. This consideration could be further ascertained from Eqn 2. The linear combination of DASA(Val-157) and V_{repul} parameters gives an almost complete regression equation of $r = 0.9965$. This equation suggests the direct participation of the papain Val-157 residue in the inhibitory activities of a series of EAAs, and the importance of hydrophobic interaction between the EAA side chain and the Val-157 residue is clearly evident. As is imaginable from Fig. 2, Val-157 is located near the position that the side chain of the EAA occupies, and therefore the proper size of the side chain is required to reveal the potential inhibitory activity.

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